# Marine Bioluminescence: Mechanisms and Evaluation

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## LONG-TERM GOALS

Our long-term goal is to understand the mechanisms and adaptive significance of marine bioluminescence (BL) and to apply knowledge thus gained towards evaluation of population dynamics of pelagic marine organisms. The ubiquity of marine BL, the huge variety of its chemical and physiological mechanisms and regulatory behavior, when compared with the present scarcity of knowledge in all these sub-disciplines, argues that marine bioluminescence most probably has major unknown significance to life in the sea (Case, et al., 1995). Moreover, experience has shown that understanding of bioluminescence as a scientific problem provides a valuable store of knowledge for naval and other applied applications.

## OBJECTIVES

Our specific objectives are expressed in three types of questions about BL involving increasing levels of complexity from cellular through populational. All have some hope of at least partial answers that appear to lie within the limits of our present resources.

## APPROACH

### Laboratory investigations

One of our central long-term goals is to try to completely characterize a bioluminescent system from stimulus to light output. No eukaryotic bioluminescent system has been fully characterized in this manner. This goal has two main elements:

(1) how luminescent cells initiate and regulate their light output, and
(2) how the luminescent organism perceives the necessity for and commands light output.

### Investigations at sea

At this level concern is with the adaptive significance of bioluminescence. While we have examined pivotal examples of adaptive significance in laboratory experiments (Harper and Case, 1999), we hope to study adaptive significance on a populational level and in the context of conventionally measurable oceanographic parameters. This approach is already beginning to be realized through application of new systems of BL measurement in the coastal environment (see this volume, Case N00014-98-1-0202)

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## WORK COMPLETED

### Luminescence excitation

Graduate student Carrie McDougall is working to elucidate the intracellular processes involved in sensing a mechanical stimulus and transducing that stimulus into a biochemical reaction resulting in light output. Cultured bioluminescent dinoflagellates are used because they are free-living single-celled organisms, making it simpler to examine intracellular transduction events. She is specifically concentrating on the role of the cytoskeleton in sensing mechanical forces. The cytoskeleton has been shown to provide structural integrity to the cell and to respond to external mechanical stimuli through redistribution and changes in concentration. Studies with mammalian cells have indicated that the cytoskeleton is an integral component of the mechanotransduction machinery. Therefore, the role of the cytoskeleton in mechanotransduction in *P. fusiformis* is being examined.

### Long-term population dynamics of luminescent plankton

### Thin Layers Investigations

All fieldwork in a study of the possible impact of bioluminescence on the biota concentrated in thin layers has been completed. Using the new small BP and a LLL-video "splatcam" (after Widder) deployed vertically through thin layers, together with simultaneous acoustic measurements of organism number and size (in collaboration with Van Holiday of BAE) evidence has been gathered in regard to the possibility that luminescence of organisms in or associated with thin layers may attract or repel zooplankters capable of visual orientation.

### Multiyear study of S. California Bight Luminescence

All fieldwork has been competed on this analysis of the population dynamics of primarily dinoflagellate luminescent sources on a transect between San Diego and San Clemente Island. This comprises the doctoral thesis of David Lapota. The 4-year study involved long term measurements from two moored bathyphotometers and periodic sampling from shipboard along the transect line from San Diego Bay to San Clemente Island. The data shows the annual succession of heterotrophic and autotrophic dinoflagellates and the effects of several environmental factors including rain runoff.

### Bioluminescence at LEO-15

A moored profiling BP and several BPs were deployed from shipboard this past summer were used to examine spatial and temporal variability of bioluminescence at this site. Data analysis has just commenced. See Moline, et al (2000) and in this volume: Moline & Case, High Resolution Temporal studies of Near Shore Vertical Structure of Bioluminescence, N00014-00-1-0008.

### Bioluminescence Short Term Predictive Study –MUSE

In August-September an array of BPs was inserted into the ongoing MUSE experiment to provide data for a bioluminescence prediction effort. The work was successfully completed and data workup is starting. SHD Haddock coordinated the BP deployments and describes the general program in his report in this volume (Zooplankton and Phytoplankton Contributors to Bioluminescence in Monterey Bay, N00014-00-1-0842)

## RESULTS

### Luminescence excitation

Quantification of filamentous actin was accomplished throughout the cell cycle. McDougall found that *Pyrocystis. fusiformis* cultures grown under continuous low-level fluid shear exhibited growth rate inhibition, cell wall size reduction, cell morphology changes, and increases in filamentous actin. Cultures were exposed to fluid shear levels below those that stimulate BL, but in the range that are found commonly in the ocean. Control cultures were grown in the same physical environment without exposure to fluid shear. The findings indicate that exposure to fluid shear can affect a cell population's ability to grow. Thus, the amount of fluid shear present in a cell's environment appears to be an important determinant in dinoflagellate cell population dynamics. The increase in filamentous actin observed in the cells exposed to shear indicates that the actin cytoskeleton responds to environmental fluid shear by rearrangement and increases in concentration. It was also found that mechanically stimulated BL output in *P. fusiformis* was increased upon depolymerizing the filamentous actin cytoskeleton. This indicates that the filamentous actin cytoskeleton is not a necessary component in BL mechanotransduction in *P. fusiformis*, but does appear to modulate the sensitivity to mechanical forces. It is hypothesized that the filamentous actin cytoskeleton is involved with providing the cell with the structural integrity necessary to resist mechanical forces, thus, decreasing the amount of filamentous actin resulted in increased sensitivity and increased BL response.

### Long-term population dynamics of luminescent plankton

The S. California Bight study clearly shows a marked seasonality in per-cell bioluminescence as well as cell numbers in *Pyrocystis noctiluca* (autotroph) and *Protoperidinium pellucidum* (heterotroph), two major elements of luminescent phytoplankton in the study area. Their luminescence is strongly associated with environmental events such as upwelling and storm runoff from land. The data analysis already shows that using chlorophyll fluorescence as a surrogate for bioluminescence is unreliable to the extent that bioluminescence might be due to heterotrophic dinoflagellates. In addition, both the LEO-15 and MUSE work clearly showed very substantial luminescence in the coastal regime due to gelatinous zooplankton and copepods.

## IMPACT/APPLICATIONS

### Luminescence excitation

The cytoskeleton is a dynamic cellular component with ability to respond to environmental stimuli quickly through control of a multitude of intracellular processes ranging from triggering BL to cell morphology and growth rate. No one has ever before examined the role of the cytoskeleton in the BL response. McDougall's findings indicate that the state and concentration of the filamentous actin cytoskeleton dramatically affects the amount of BL triggered by fluid shear. Thus, it would appear that the filamentous actin cytoskeleton is involved in regulating the sensitivity to mechanical forces and may be able to change the threshold for stimulating BL in dinoflagellates. However, the filamentous actin cytoskeleton does not seem to be a necessary component of the mechanotransduction machinery, but rather functions as a modulating element to the sensitivity of mechanical forces. Additionally, because it was found that the amount of filamentous actin increased in response to constant low-level shear, it would appear that the cytoskeleton has the ability to respond over a few cell cycles to environmental stimuli by changing its concentration, providing the cell with increased structural

integrity to resist these forces. This seems appropriate in view of the high variable levels of mechanical excitation that dinoflagellates experience in nature as dwellers near the sea surface.

## RELATED PROJECTS

1. Collaboration with Dr. Michael Latz and his group at Scripps Institution of Oceanography, San Diego, CA., in study of the response of dinoflagellates to very low shear forces and their effect on the actin cytoskeleton in dinoflagellates.

2. Collaboration with Prof. Mark Moline, California Polytechnic University, in development of a bathyphotometer system for year-round profiling of bioluminescence at the LEO-15 Site.

3. Collaboration with Dr. David Lapota, SPAWARS, San Diego, CA., in analysis of long term bioluminescence data from the Southern California Bight.

4. Collaboration with Dr. SHD Haddock, MBARI, Moss Landing, CA. in investigation of distribution of marine bioluminescent organisms in the coastal zone.

5. Collaboration with Dr. S Gaines, et al, A Consortium of Excellence in Marine Conservation (Packard Foundation) and Dr. D. Reed, et al, Land/Ocean Interactions and Dynamics of Kelp Forest Ecosystems (NSF, LTER), both of UCSB. These two programs have long-term instrumented moorings in the coastal zone from Santa Barbara to N. of Pt. Conception. We plan to install at least three of our BPs on these mooring to continue our coastal investigations.

## REFERENCES

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